# Human Nasal Mucosal Changes after Exposure to Urban Pollution

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Millions of people worldwide are living in areas where ozone ($O_3$) concentrations exceed health standards (an hourly average of 235 μg/m$^3$/0.12 ppm, not to be exceeded more than once per year). Ozone induces acute nasal inflammatory responses and significant epithelial lesions in experimental animals and humans. To determine the nasal effects of a 15-day exposure to an urban polluted atmosphere with $O_3$ as the main pollutant, we studied a population of healthy, young males newly arrived to southwest metropolitan Mexico City (SWMMC). The study included 49 nonsmoking residents in an unpolluted port, Veracruz City; 14 subjects stayed in the port and served as controls, while 35 subjects traveled to SWMMC and had serial nasal lavages at different times after arriving in SWMMC. Subjects had exposures to ambient $O_3$ an average of 10.2 hr/day, with a total cumulative $O_3$ exposure of 10.644 ppm•hr. Nasal inflammatory responses, polymorphonuclear leukocyte PMN-CD11b surface expression, rhinoscopic changes, and respiratory symptoms were evaluated.

Exposed subjects had massive nasal epithelial shedding and significant responses in PMN nasal influx ($p<0.00001$) and in PMN-CD11b expression ($p<0.05$). Cumulative $O_3$ exposure correlated with respiratory symptoms, PMNs ($r_s = 0.2374$, $p<0.01$), and CD11b ($r_s = 0.3094$, $p<0.01$); 94% of exposed subjects experienced respiratory symptoms, and 97% left the city with an abnormal nasal mucosa by rhinoscopy. Nasal epithelial changes persisted 2 weeks after the exposed subjects returned to their nonpolluted environment. Exposure to an urban polluted atmosphere induces significant and persistent nasal epithelial alterations in healthy subjects. Because $O_3$ is the main pollutant for SWMMC and concentrations of other pollutants (e.g., sulfur dioxide, nitrogen dioxide, total suspended particulates, formaldehyde) were well below the standard levels or undetectable, we suggest that $O_3$ is likely to play a role in the etiopathogenesis of the nasal alterations along with the effects of other atmospheric pollutants which were not measured. *Key words*: adhesion molecules, air pollution, human nasal mucosa, nasal lavage, ozone. *Environ Health Perspect* 102:1074–1080 (1994)

Ozone ($O_3$) is a major constituent of photochemical smog, and millions of people worldwide are exposed recurrently to $O_3$ concentrations at and above the current National Ambient Air Quality Standard (NAAQS) for $O_3$ (0.12 ppm as a 1-hr maximum concentration, not to be exceeded more than once per year), while millions more with exposures to lower concentrations have large cumulative $O_3$ exposures over time. A number of respiratory health effects from $O_3$ exposures have been well documented both in experimental animals and humans (*1*).

The nose, a highly complex organ, is the portion of the respiratory tract first in contact with inhaled gases, including $O_3$, which it removes from inspired air with varying degrees of uptake efficiency (*2*). Bonnet monkeys exposed to 0.15 ppm $O_3$ for 6 days show nasal ciliated-cell necrosis, shortened cilia, and a marked inflammatory cell influx (*3*). Studies in rats (*4*) suggest that $O_3$ is capable of rapidly inducing hyperplastic and metaplastic responses in the nasal nonciliated cuboidal epithelium and that once initiated, development of phenotypic changes within the epithelium does not require further $O_3$ exposure.

Human nasal responses to $O_3$ exposure have been investigated in control chamber studies (*5–7*). Although these acute studies provide valuable information, they may not reflect changes in the nasal passages of subjects exposed to continuous, ambient $O_3$ for several hours a day for many days and weeks. This situation is likely for people spending most of their daylight time outdoors, when $O_3$ concentrations follow distinct patterns characterized by daylight peaks with levels both above and below the NAAQS.

We previously reported that subjects who have resided in southwest metropolitan Mexico City (SWMMC) for less than 30 days display loss of normal nasal ciliated-type epithelium, basal cell hyperplasia, and mild epithelial dysplasias, as evaluated by nasal turbinate biopsy (*8*). To achieve a better understanding of the nasal pathological responses of newly exposed subjects to a polluted urban atmosphere with $O_3$ as the predominant criteria pollutant, we investigated 1) the severity of the nasal surface epithelial damage to increasing, cumulative outdoor exposure times, as evaluated by cellular changes detected in nasal lavage samples, 2) whether nasal responses and clinical respiratory symptomatology correlate with concentration and time of exposure to $O_3$ as evaluated by fixed-site $O_3$ measurements, and 3) whether the $O_3$-induced nasal injury that occurred during the 15-day polluted atmosphere exposure persisted until 2 weeks of "recovery" in a markedly less polluted environment.

## Methods

***Study population.*** This project was approved by the Instituto Nacional de Pediatria Review Boards for Human Studies, and informed written consent was obtained from all subjects. The 49 participants were permanent Veracruz residents, had never been to Mexico City, and seldom left their city of residence. These subjects were healthy, nonsmoking males, average age 23.5 ± 4.2 years, with an outdoor exposure time of 10 ± 3 hr per day, engaged in security duties and used to a daily work routine with moderate to heavy exercise outdoors. Thirty-five of these subjects traveled to SWMMC in a work-related trip, and the remaining 14 subjects stayed in Veracruz and served as controls. Clinical data obtained included age, place and length of residence, occupational history, history of toxic exposures, allergic diseases, smoking and drinking habits, otolaryngological history (epistaxis, quantity and quality of nasal mucus, nasal dryness, nasal obstruction, rhinorrhea), and respiratory symptomatology (cough, thoracic pain, and dyspnea). Potential subjects were excluded from participation if they had ever smoked or had been exposed to environmental tobacco smoke in the year previous to the study, had a history of asthma, allergic or infectious rhinitis, recent acute respiratory illness (in the previous 6 months), chronic respiratory disease, or exposure to toxic substances (e.g., paints, solvents, wood dust, metals, photocopying machines). None of the 49 volunteers showed any evidence in their medical

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We are indebted to Sergio Gomez-Llata for his invaluable help and support of this project. Special thanks to Humberto Bravo and his staff for kindly letting us access the atmospheric data from the Unidad de Contaminación Ambiental, Centro de Ciencias de la Atmósfera, UNAM; Guadalupe Roy-Ocotla for atmospheric data analysis; Anna and Jessica Villarreal for their assistance in collecting clinical data; Humberto Garnica Ornelas for the art work; and Ana María Martínez for secretarial support. We are grateful to all the volunteers who took part in the study.

Received 10 June 1994; accepted 31 August 1994.

histories or after complete physical examination of any viral illness or respiratory disease.

***Follow-up schedule and outcome of measurements.*** All enrolled subjects had a clinical history and a physical examination including ear–nose–throat exams at day 1. The control subjects of the study ($n$ = 14) were examined in Veracruz 1 week before the exposed subjects and again at the 2-week post-return visit ($n$ = 8). The 35 participants from the exposed group traveled from Veracruz to Mexico City, arrived at 2000 hr and were transported to their SWMMC quarters; they were seen by researchers the following day at 0800 hr. Their complete work up was done on their first day in the city. Thereafter, these subjects were seen at 36, 42, 90, 132, 180, 256 and 300 hr after arriving in SWMMC. Otorhinolaryngological and respiratory symptomatology were recorded at each visit, and a nasal lavage (NL) sample was taken. One their 13th day in the city, a complete physical examination and an ear–nose–throat examination were performed. Two weeks after their return to Veracruz, the last NL sample was taken from both control and exposed subjects. The control subjects had 22 NL samples from two sampling dates, and the exposed subjects had 175 NL samples from 9 sampling times. The SWMMC-exposed volunteers were divided from the beginning of the study into 2 subgroups: A ($n$ = 17) and B ($n$ = 18), in order to take NL samples the same day (i.e., A1 and B2) or closely adjacent days (i.e., A3 and B4) (see Table 1), since nasal lavage may alter the cellular populations in the nasal mucosa, and a "washout" effect may persist for several hours (*7*).

The SWMMC NL samples were taken in the morning (0715–0900 hr) or afternoon (1200–1400 hr), except for the first sampling day when subjects in subgroup A had their NLs done by 1000 hr and subjects in subgroup B had theirs between 1100 and 1300 hr. For the clinical evaluation analysis, we grouped all subjects and present the data for the following periods: 12, 42, 132, 256, 300 hr, and 2-week post-return to Veracruz. The SWMMC subjects were housed in well-ventilated buildings that used open windows for cooling; no local sources of toxic substances were identified (including nitrogen oxides, paints, solvents, cleaning materials, and smoke). The living–working quarters had low-density housing and the premises were enclosed, so vehicular traffic was minimal. All exposed subjects had the same work routine [stayed outdoors from 0630 hr to 1800 hr with only brief intermittent periods indoors (less than 1 hr total]. Six days per week, from 0800 to 1300 hr subjects exercised moderately outdoors (ventilation during exercise, $V_E$ = 37–39 l/min, data not shown), and had intermittent, light exercise the remainder of the daylight time. Control subjects followed an identical work routine which included moderate daily exercise outdoors.

***Nasal lavage procedure.*** The NL procedure used in this study was adapted from Koren et al. (*7*). Sterile phosphate-buffered saline (PBS, 10 ml) without $Ca^{2+}$ and $Mg^{2+}$ at 37°C was instilled, 5 ml in each nasal cavity, using a blunt-tipped syringe while the subjects were seated with their heads reclined at a 45° angle. The saline was held in the nasopharyngeal region for 10 sec and then forcibly expelled into a sterile plastic specimen cup, and the nasal passages were allowed to drain into the cup for 30 sec. The volume of lavage fluid recovered was recorded and used to adjust the cell counts by volume, which were expressed as cells per milliliter of recovered NL fluid. The samples were centrifuged at 1000g for 15 min, and the supernatant was separated and frozen at -70°C for subsequent analysis. The NL cell pellet suspended in 1 ml of PBS was used for the hemocytometer total cell counts, cytocentrifuge slide preparations, and for flow cytometer studies. Cytocentrifuge-prepared slides were stained with Papanicolau and Wright-Giemsa stains before counting. We examined 200 cells under oil immersion. We used the differential cell count to calculate PMNs per milliliter of recovered lavage fluid. Papanicolau-stained slides were used for cytological evaluation.

***Flow cytometric analysis.*** Anti-Mac 1 (CD11b) (clone 2 LPM 19C) was purchased from Dako Corp. (Carpintería, California). The isotypic control, phycoerythrin-matched Ms IgG1 was from Coulter Corp. (Hialeah, Florida).

Expression of CD11b/CD18 on the surface of nasal PMNs was measured on a fluorescence-activated cell sorter flow cytometer (EPICS-Profile II, Coulter). In brief, $5 \times 10^5$ single NL cells suspended in 100 µl of PBS without $Ca^{2+}$ and $Mg^{2+}$ were incubated at room temperature (15°C) for 10 min with either the specific mAb or the isotype-matched control mAb (10 µl of the undiluted mAb). After incubation, we washed the cells and immediately analyzed them by FACS. From each sample assayed in duplicate, 5000 gated events were collected, and the mean fluorescence intensity was measured. Nonspecific fluorescence was determined on cells incubated with a mouse IgG of the same isotype as the mAb but with irrelevant antigen specificity. We determined the mean specific fluorescence by subtracting the measured nonspecific fluorescence from the mean fluorescence for the studied population. The PMN population was identified by its forward light scatter and side light scatter characteristics. The cursor was set so that less than 2% of the cells in each sample stained positively with the negative control sample. The percentage of cells that stained positively and the mean fluorescence intensity were recorded for each sample. The results were displayed as single-parameter histograms with the X-axis representing the $\log_{10}$ of the relative fluorescence intensity per cell and the Y-axis representing the number of cells. Peak fluorescence of each histogram was also converted to a linear number for tabular display.

***Pollutant methodology.*** Atmospheric pollutants and meterological conditions were monitored at the University Station located in SWMMC, downwind of the major diurnal emissions in metropolitan Mexico City and 1.3 miles from the SWMMC volunteers quarters. Ozone was monitored using a Beckman 950 chemiluminescence analyzer with a calibration routine in accordance with U.S. EPA procedures. We measured the area under the curve for $O_3$ concentrations from 0630 to 1800 hr and expressed the results as $O_3$ concentrations × time ($O_3$ ppm•hr). Analysis of formaldehyde was made by the chromotropic acid sulfuric method, and the observance of the colored solution was read

**Table 1.** Characteristics of the southwest metropolitan Mexico City-exposed subgroups in terms of nasal lavage (NL) sampling times, cumulative $O_3$ exposure, and maximum $O_3$ peaks

| Sample | NL sampling time (hr) | $O_3$ (ppm•hr) | $O_3$ maximum peak | Time of NL |
|---|---|---|---|---|
| A1 | 12 | 0.068 | 0.022 | AM |
| A3 | 36 | 1.135 | 0.167 | AM |
| A5 | 90 | 3.379 | 0.189 | PM (0.689 ppm•6 hr) |
| A8 | 180 | 5.898 | 0.094 | AM |
| B2 | 17 | 0.450 | 0.134 | AM/PM |
| B4 | 42 | 1.301 | 0.199 | PM (0.514 ppm•5 hr) |
| B6 | 132 | 4.633 | 0.128 | AM |
| B8 | 256 | 8.128 | 0.225 | PM (0.780 ppm•6 hr)[a] |
| A9 | 300 | 9.672 | 0.082 | AM |
| B9 | 300 | 9.672 | 0.082 | |
| Departure day 15 | 348 | 10.644[b] | 0.074 | |

[a]Highest $O_3$ exposure previous to a nasal lavage exam.
[b]Cumulative $O_3$ exposure (C × T, ppm•hr) in the 15-day period in SWMMC.

from an SGP-350 visible spectrophometer PYE UNICAM at 580 nm. Temperature, relative humidity, wind speed, and rain events were also monitored. Data from Veracruz were obtained from the Centro de Previsión del Golfo de Mexico.

***Statistical analyses.*** Data capture rate was 100% for all SWMMC participants. We analyzed results in three ways on an IBM PC computer using a software package (PAQUEST VI-0; Biomedical Software Developments, Mexico): nonparametric Kruskal-Wallis test to compare PMNs and CD11b control values with exposed samples, nonparametric analysis by ranks Friedman's test for the differences in PMNs and CD11b values between the six sampling dates in each of the exposed subgroups; and correlation coefficients ($r$) to correlate cumulative $O_3$ ppm•hr and clinical symptoms. Spearman's correlation coefficients were calculated for PMNs and CD11b values and cumulative $O_3$ exposures; $p$ values <0.05 were considered significant (*9,10*).

## Results

***Atmospheric pollutants and meterological characteristics.*** The 35 SWMMC volunteers were exposed to atmospheric $O_3$ an average of 10.2 hr per day; $O_3$ became detectable by 0800–1000 hr and remained elevated until 1900 hr. Table 1 shows the cumulative $O_3$ exposures and the maximum $O_3$ peaks. On the day subjects left SWMMC, the cumulative $O_3$ exposure was 10.6 ppm•hr; the maximum $O_3$ peak recorded was 0.225 ppm, and the average daily maximum $O_3$ concentration was 0.171 ppm. Interestingly, if we only took into account the number of hours with $O_3$ above the NAAQ standard, the daily average was 2.6 hr with a range of 0–6 hr.

The highest $O_3$ exposure previous to an NL sample was 0.780 ppm in 6 hr for sample B8 taken in the afternoon (Table 1). Formaldehyde concentrations for the study period were below 0.005 ppm (13.8 $\mu g/m^3$) for the atmospheric samples taken hourly between 0800 and 1300 hr. $SO_2$, $NO_2$, total suspended particles, and $PM_{10}$ were below EPA air quality standards. Ambient outdoor temperature ranged from 13° to 26°C and relative humidity from 40 to 97%. Veracruz meteorological conditions for the two sampling dates were average for the season: 25°C, relative humidity 97% and northeast winds at 20 km/hr.

***Clinical assessment.*** The age and clinical characteristics of the 49 volunteers were similar. None of the subjects complained of respiratory symptoms while living in Veracruz, except in the event of an upper respiratory infection (on average two infections per year). However, SWMMC-exposed subjects had respiratory complaints as early as 12 hr after arrival (Table 2; Fig. 1) and by the time subjects left the city, 94% had respiratory complaints. We found high correlation coefficients between certain respiratory symptoms and cumulative $O_3$ exposure: rhinorrhea, $r$ = 0.97; nasal mucus, $r$ = 0.96; nasal obstruction, $r$ = 0.93; and cough with exercise, $r$ = 0.88. The correlation was lower for epistaxis ($r$ = 0.30), but interestingly, the period of time with the highest number of subjects with epistaxis (132 hr) coincides with NL sample B6 ($n$ = 18; Table 1), which shows a high correlation coefficient between NL CD11b and PMNs ($r$ = 0.62, $p$<0.01). Thoracic pain reached its maximum at 42 hr, but remained present in one-third of the volunteers until departure time. Two weeks after their return to Veracruz, five subjects (14%) had persistent complaints: nasal obstruction ($n$ = 5), nasal mucus ($n$ = 4), rhinorrhea ($n$ = 3), and one subject complained of nasal dryness and sporadic thoracic pain. None of the exposed subjects had any clinical evidence of a viral illness or respiratory disease throughout the study period.

***Rhinoscopy findings.*** Although all control subjects had a normal nasal mucosa by direct rhinoscopic exam, 31% of exposed subjects had a hyperemic or a pale mucosa 15 hr after their arrival and by day 13. All but one subject exhibited a nasal mucosa with bilaterally irregular patches of opaque, pale, thin areas in the inferior and middle turbinates.

***Cytocentrifuge nasal lavage samples.*** Cytocentrifuge NL samples from controls and 12-hr exposed samples contained a few epithelial cells, mostly squamous cells (Fig. 2A); by 42 hr ciliated respiratory-type cells were recovered and showed loss of cilia and small clear cytoplasmic vacuoles (Fig. 2B).

Massive epithelial shedding was a major finding from 132 hr onward (Fig. 2C); squamous cells, basal cells and ciliated type cells showed nuclear pyknosis and karyorrhexis with numerous cytoplasmic vacuoles and acidophilic inclusions (Fig. 2D). PMNs were abundant in samples taken from 132 hr onwards (Fig. 2E), coinciding with the presence of ghost epithelial cells and cellular debris. Intact red blood cells could be seen in all samples from 36 hr onward, but were particularly visible in samples at 132 and 180 hr. Macrophages, some with hemosiderin granules, were present as early as 90 hr. The cytocentrifuge samples taken 2 weeks after the subjects returned to Veracruz showed persistent PMNs and cellular debris, with a few epithelial cells displaying conspicuous nuclei (Fig. 2F).

***Nasal lavage PMNs and PMN-CD11b expression.*** There were no differences in

**Table 2.** Respiratory symptomatology in the southwest metropolitan Mexico City-exposed subjects

| | % with symptoms | | | | | |
|---|---|---|---|---|---|---|
| Symptom | 12 hr | 42 hr | 132 hr | 256 hr | 300 hr | Veracruz, 2 weeks |
| Rhinorrhea | 20 | 20 | 28.57 | 48.57 | 60 | 8.57 |
| Cough with exercise | 20 | 28.57 | 28.57 | 37.14 | 34.28 | 0 |
| Nasal dryness | 14.28 | 40 | 68.57 | 71.42 | 88.57 | 2.85 |
| Nasal mucus | 14.28 | 20 | 48.57 | 51.42 | 60 | 11.42 |
| Nasal obstruction | 8.57 | 20 | 40 | 37.14 | 54.28 | 14.28 |
| Epistaxis | 5.71 | 8.57 | 28.57 | 20 | 8.57 | 0 |
| Thoracic pain | 5.71 | 42.85 | 20 | 34.28 | 34.28 | 2.85 |
| No. of subjects with symptoms | 16 | 29 | 32 | 33 | 33 | 5 |
| % of total subjects | 46 | 83 | 91 | 94 | 94 | 14 |

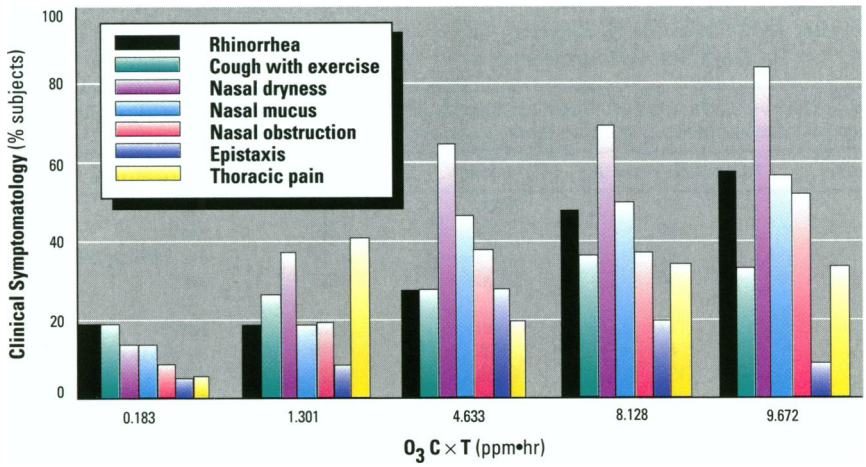


**Figure 1.** Respiratory symptoms and their relationship to cumulative $O_3$ exposure (ppm-hr) in the southwest metropolitan Mexico City-exposed subjects. Clinical evaluation data correspond to 12, 42, 132, 256, and 300 hr in southwest metropolitan Mexico City.

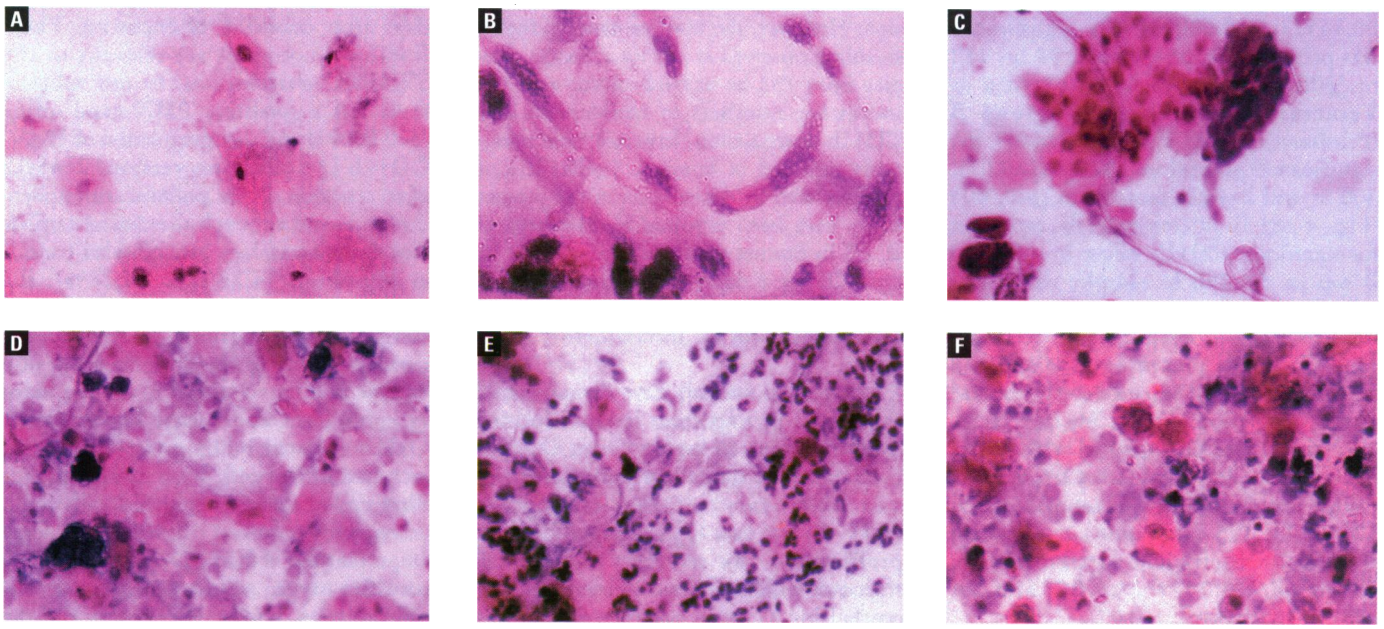


**Figure 2.** Cytocentrifuge Papanicolau-stained preparations obtained from the nasal lavage of a 24-year-old southwest metropolitan Mexico City-exposed subject. (A) Squamous cells against a clean background; 12-hr sample, ×160. (B) Ciliated respiratory-type cells in profile. Note the thin, whiplike, elongated proximal ends and cytoplasmic vacuolization; 42-hr sample, ×400. (C) Sheets of squamous cells and small basal cells along with hemosiderin-laden macrophages; 132-hr sample, ×160. (D) Heavy exfoliation of epithelial cells, cellular debris, bizarre shapes of ghost cells; nuclei show pyknosis and karyorrhexis, accounting for the irregularity of the nuclear outline; 256-hr sample, ×160. (E) Numerous neutrophils with cellular debris, cell ghosts, and multinucleated macrophages; 300-hr sample, ×160. (F) Specimen taken 2 weeks post-return to Veracruz; there are persistent polymorphonuclear leukocytes and cellular debris; a few epithelial cells display conspicuous nucleoli, ×160.

the volume of NL fluid recovered from control and exposed subjects (7.9 ± 1.2 ml). Table 3 shows the median, maximum, and minimum values for PMNs per milliliter and CD11b for the controls and the two exposed subgroups. The PMN control values were significantly different from the exposed group at 36 hr (Kruskal-Wallis; 36 hr, $p<0.001$; 42 hr, $p<0.01$; 132 hr, $p<0.05$; and 2 weeks post-return to Veracruz, $p<0.00001$). CD11b values were significantly different from the control group at 300 hr ($p<0.05$).

We then compared the exposed subjects using the 12 hr (subgroup A) and the 17 hr (subgroup B) PMNs and CD11b values as the baseline and comparing these values with the remaining sampling times using the Friedman's test. For subgroup A, PMN values were statistically significant (Friedman $\chi^2 = 23.95881$, $p = 0.0002$), and as shown in Figure 3A, the significance was for samples at 36 hr ($p<0.05$), 180 hr ($p<0.01$), and 2 weeks post-return to Veracruz ($p<0.01$). For subgroup B, PMN values were also statistically significant (Friedman $\chi^2 = 41.923651$, $p<0.0001$), and Figure 3B shows the significance for samples at 42 hr ($p<0.05$), 132 hr ($p<0.001$), 256 hr ($p<0.01$), and 2 weeks post-return to Veracruz ($p<0.001$). The low PMN values for samples at 36 and 42 hr are probably the result of a wash-out effect (*7*), since the NL samples were taken 24 and 25 hr apart, respectively.

CD11b values for subgroup A were statistically significant (Friedman's $\chi^2$ =

**Table 3.** Polymorphonuclear leukocyte (PMN) and CD11b values for controls and southwest metropolitan Mexico City-exposed subjects

| Sample | Hours in city | No. of subjects | PMN/ml | | | CD11b[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | Median | Max | Min | Median | Max | Min |
| Controls | 0 | 14 | 8435 | 16,870 | 4380 | 6.7 | 7.814 | 2.886 |
| A | 12 | 17 | 3440 | 34,230 | 1320 | 4.27 | 9.56 | 1.785 |
| A | 36 | 17 | 1760 | 21,670 | 1000 | 5.71 | 16.37 | 0.880 |
| A | 90 | 17 | 5670 | 42,300 | 1600 | 5.88 | 22.38 | 3.224 |
| A | 180 | 17 | 11,920 | 175,070 | 1840 | 13.68 | 39.37 | 1.885 |
| A | 300 | 17 | 7760 | 262,920 | 1580 | 10.52 | 30.28 | 2.499 |
| A | 0[b] | 17 | 20,930 | 62,970 | 5810 | 6.13 | 25.55 | 3.606 |
| B | 17 | 18 | 4270 | 119,500 | 1350 | 3.17 | 9.783 | 1.277 |
| B | 42 | 18 | 1710 | 72,270 | 1360 | 5.21 | 15.88 | 1.341 |
| B | 132 | 18 | 19,725 | 347,220 | 2140 | 7.31 | 24.61 | 1.711 |
| B | 256 | 18 | 7975 | 99,700 | 1940 | 4.75 | 34.61 | 1.726 |
| B | 300 | 18 | 4120 | 35,410 | 1460 | 7.54 | 39.63 | 1.15 |
| B | 0[b] | 18 | 22,025 | 83,420 | 10,510 | 8.11 | 16.76 | 3.566 |
| Controls | 0 | 8 | 6340 | 12,020 | 3520 | 6.1 | 7.73 | 2.224 |

[a]CD11b values expressed as mean fluorescence intensity.
[b]Nasal lavage samples of exposed subjects taken after 2 weeks in Veracruz.

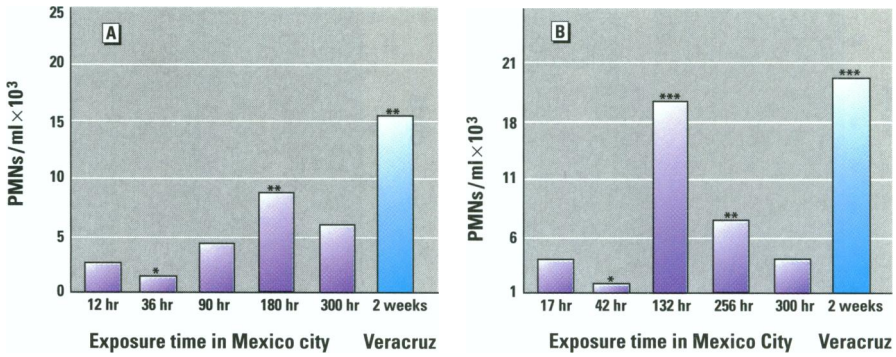


**Figure 3.** Nasal lavage polymorphonuclear leukocytes (PMNs) per milliliter median values for the exposed subgroups A and B. (A) For subgroup A, PMN values significantly different from the 12-hr sample at 36 hr, 180 hr, and 2 weeks post-return to Veracruz (Friedman's test). ) (B) For subgroup B, significance found at 42, 132, 256 hr, and 2 weeks post-return to Veracruz. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

30.44511, $p<0.00001$). The significance was present at 36 hr ($p<0.05$), 90 hr, 180 hr, 300 hr and 2 weeks post-return to Veracruz ($p<0.001$) for subgroup B (Friedman's $\chi^2 = 21.07938$, $p<0.0001$), the significance present at 132 hr ($p<0.01$), 300 hr and 2 weeks post-return ($p<0.001$) (Figs. 4 and 5). Interestingly, the exception for significant CD11b values in subgroup B was in the 256 hr sample, which was taken in the afternoon immediately after the subjects had been exposed to 0.780 ppm $O_3$ in the preceding 6 hr and had performed sustained, moderate exercise during this period of time. We then correlated cumulative $O_3$ (ppm•hr) with PMNs per milliliter and CD11b values, and we found for the SWMMC samples (1–9, 12–300 hr, 175 data points) a significant correlation coefficient (Spearman) for PMNs ($r_s = 0.2374$, $p<0.01$) and for CD11b ($r_s = 0.3094$, $p<0.01$).

## Discussion

The results of this study demonstrate that relatively short exposures to a polluted urban atmosphere can produce important nasal epithelial pathology in healthy, young humans. The SWMMC atmosphere is characterized by high levels of photochemical smog, with $O_3$ levels above the NAAQS throughout the year, while other criteria pollutants are below standards or in background concentrations (e.g, formaldehyde). SWMMC-exposed subjects showed a progressive pattern of ciliated-type respiratory cell damage detected 42-hr after arrival in the city and before the inflammatory cell influx. This early nasal epithelial necrosis might be the result of direct $O_3$ toxicity, and neutrophils could contribute to the injury at later time points.

These observations are similar to those of Pino et al. (*11*), where rats exposed to 1.0 ppm $O_3$ for periods between 4 and 24 hr showed early epithelial cell necrosis in terminal bronchioles before PMN migration. However, the massive nasal epithelial shedding observed in the nasal lavage samples from 132 hr onward, along with the increasing numbers of neutrophils and CD11b upregulation, have not been previously reported in humans. Similar lesions to those seen in the subjects in the present study have been reported by Harkema et al. (*3*) in Bonnet monkeys exposed to 0.15 ppm $O_3$ for 6 days; these animals had an increased number of necrotic nasal ciliated cells along with an inflammatory nasal influx.

Studies in animals have demonstrated that the most important lesions resulting from inhalation of high ambient $O_3$ concentrations are located in the nasal cavity and pulmonary centriacinar regions (*3,11–19*). In rats, $O_3$ can rapidly induce proliferative and secretory metaplastic responses within nasal nonciliated cuboidal epithelium (*4*) and produce significant changes in the stored nasopharyngeal epithelial mucoid substances (*17*). Ozone concentrations as low as 0.15 ppm can induce significant epithelial lesions in the macaque proximal respiratory bronchioles after both short-term (6 days, 8 hr per day) and long-term (90 days, 8 hr per day) exposures (*13*). An important issue in short-term exposure to high ambient ozone concentrations is that once the $O_3$-induced injury is initiated, the development of $O_3$-induced epithelial damage does not depend on further $O_3$ exposure, a likely situation in our SWMMC-exposed individuals and an issue dealt with by Hotchkiss et al. (*4*) for $O_3$-exposed rats. These authors demonstrated that $O_3$ can rapidly induce phenotypic changes in the nasal nonciliated cuboidal epithelium and that once initiated, development of these epithelial changes does not require further $O_3$ exposure. Harkema et al. (*17*) demonstrated that after a 7-day exposure to 0.12 ppm $O_3$, the increase in stored mucoid substances in the nasal rat turbinates persisted for at least 7 days after cessation of exposure. These findings in animals are relevant to the SWMMC observations. In our subjects there was a persistent PMN nasal influx ($p<0.00001$) and an upregulation of PMN-CD11b ($p<0.001$) expression, 2 weeks after cessation of the polluted atmosphere exposure, and although the clinical respiratory symptomatology had subsided considerably (from 94.28% to 14.28%), the abnormal nasal rhinoscopic and cytocentrifuge findings remained. These abnormal persistent findings could be the result of a cascade of events initiated in the respiratory epithelium upon acute $O_3$ exposure. Oxidant-related injury and activation of recruited PMNs with acute damage to capillary endothelial and epithelial cells could account for the nasal epithelial pathology. Important too is the PMN's ability to generate free oxygen radicals and produce neutral proteases, lysosomal enzymes, and the products of endogenous arachidonic acid metabolism.

Stimulated endothelial and epithelial cells produce inflammatory mediators and cytokines that induce the expression of different adhesion molecules. CD11b, a member of the $ß_2$ subfamily of human leukocyte integrins, is essential for adhesion-dependent granulocyte functions such as phagocytosis, chemotaxis, and aggregation and is also a receptor for iC3b, an opsonic fragment of the third component of complement (*20–24*). Stimuli that increase the CD11b surface PMN expression include eicosanoids, a potent group of chemical mediators, products of airway epithelial cells after exposure to $O_3$. McKinnon et al. (*25*) have demonstrated that exposure of a human bronchial epithelial cell line BEAS-S6 to different $O_3$ concentrations releases LTC4, LTB4, LTD4, prostaglandin $E_2$, and thromboxane $B_2$.

CD11b upregulation in the $O_3$-exposed nasal lavage PMNs is an interesting but not an unexpected finding in view of the literature previously mentioned (*20–26*). CD11b is upregulated in inflammatory neutrophils *in vivo* in a matter of minutes due to translocation of preformed molecules contained in intracellular granules to the cell surface (*24*). Two observations in this regard are interesting: first, epistaxis in this study coincided with a strong correlation between PMN numbers and significant CD11b values ($r = 0.62$, $p<0.01$) and is likely an indicator of endothelial damage. Although epistaxis was a transient sign in almost one-third of exposed adults, it is present in 55% of SWMMC preadolescents that also show a significant upregulation of their nasal PMN-CD11b (Calderon-Garcidueñas L, personal observation). Second, it is conceivable that if appropriate stimuli (e.g.,

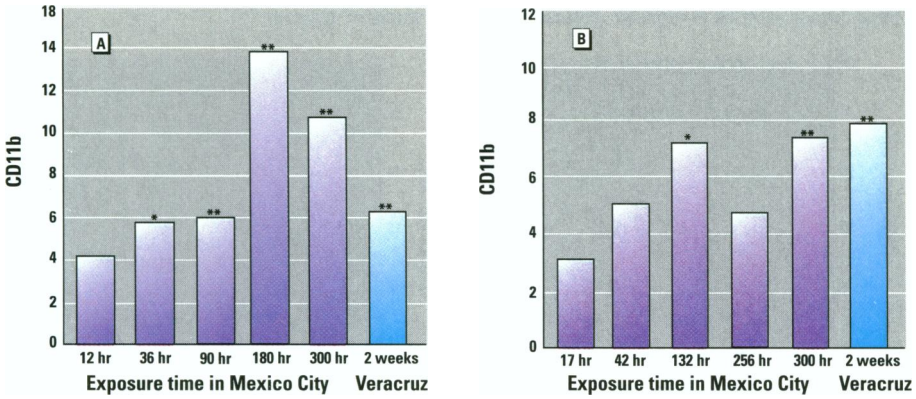


**Figure 4.** Nasal lavage CD11b median fluorescence intensity values for exposed subgroups A and B. (A) Subgroup A: CD11b values are significantly different from the 12-hr sample at all subsequent sampling times (Friedman's test). (B) Subgroup B, significant at 132 hr, 300 hr, and 2 weeks post-return to Veracruz. *$p<0.05$; **$p<0.001$.

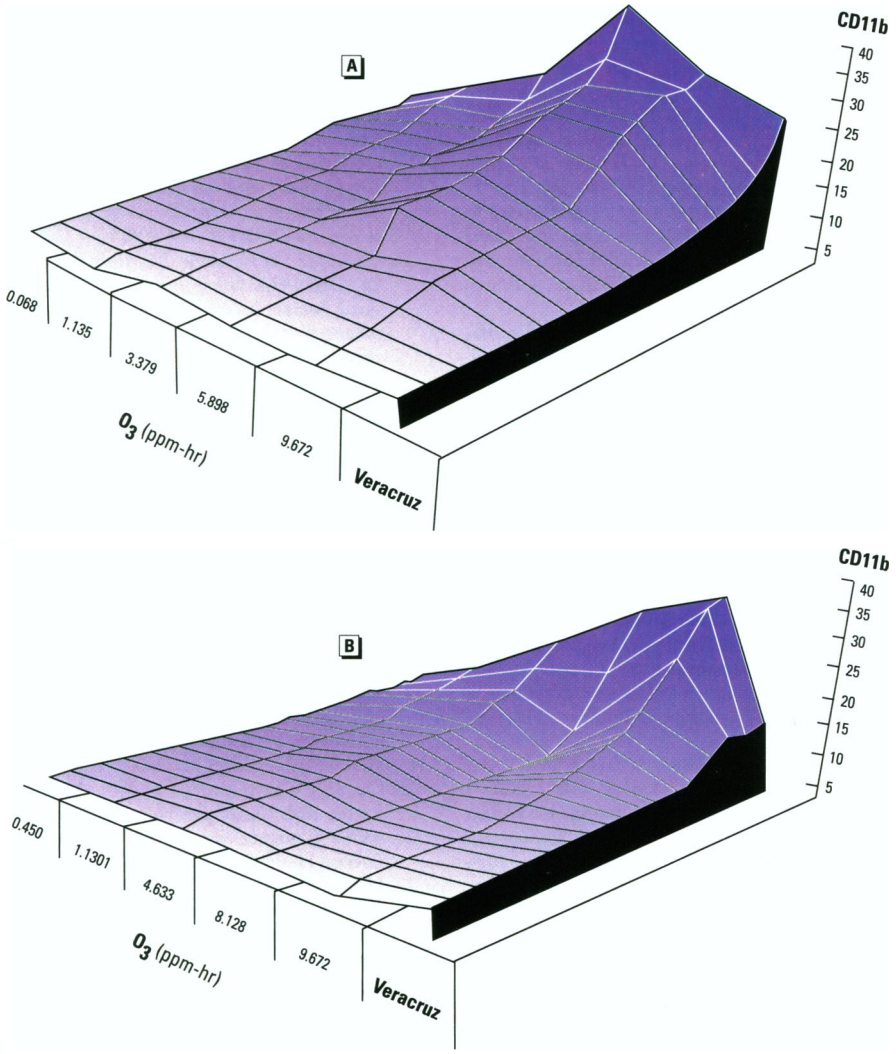


**Figure 5.** Mean CD11b fluorescence intensity values and their relationship to cumulative $O_3$ exposure (ppm•hr) for southwest metropolitan Mexico City-exposed subjects in subgroup A (A) and subgroup B (B). Notice the different cumulative $O_3$ exposures for each subgroup, corresponding to different sampling times and the depression in the CD11b values at 8.128 ppm-hr $O_3$ in subgroup B (256-hr sample B8).

$O_3$) are supplied continuously, the triggering of CD11b molecules can slowly assume a desensitized state and no longer respond with upregulation, a situation that could explain the changes seen in NL sample B8 (256 hr), taken after the volunteers were exposed to 0.780 ppm•hr $O_3$ in a 6-hr period under continuous, moderate exercise. There has been some controversy regarding the effect upon the respiratory epithelium of cumulative $O_3$ exposures versus peak exposures versus a low level background exposure. Chang et al. (*19*) suggested that alveolar epithelial cell reactions to low-level subchronic exposures to $O_3$ are directly related to the cumulative oxidant concentration. Henderson et al. (*12*), evaluating the effect of cumulative $O_3$ exposure on nasal responses in female F344/N rats, concluded that the response through DNA synthesis of the nasal epithelium was approximately equal for equal cumulative exposures if these $O_3$ exposures were >0.12 ppm. On the other hand, Harkema et al. (*13*) suggested a threshold for epithelial bronchiolar Bonnet monkey $O_3$-associated pathology and a lack of correlation between bronchiolar damage and $O_3$ concentrations or exposure times.

Based on our experience with nasal mucosal pathology in Mexico City inhabitants (*8,27*), we believe that for newly arrived populations, cumulative $O_3$ exposures are more relevant to nasal pathology than peak concentrations or hours above the NAAQS, and we fully agree with Lippman (*1*) that the current 1 hr-120 ppb $O_3$ NAAQS does not protect against short-term effects. We would like to add that at least for nasal $O_3$ effects, humans lack the development of potentially protective mechanisms described for animals, such as secretory metaplasia and increased amounts of intraepithelial mucoid substances (*4,17*), and these type of $O_3$-responses should be kept in mind when assessing data derived from $O_3$-exposed experimental animals for the purpose of assessing human risk.

In this study there was a significant correlation between $O_3$ concentrations and exposure time with respiratory symptomatology, PMN, and CD11b values. Volunteers remained outdoors an average of 10.2 hr per day, had living-working quarters with low-density housing, low traffic density, and no other pollutant sources. We are aware that personal $O_3$ monitors are better than fixed-site measurements (*28*), but lack of resources prevented their use. However, we are of the opinion that with the outdoor exposure patterns described for these subjects, ambient $O_3$ fixed-site data are helpful for correlation with clinical and pathological effects.

In summary, studying natural populations for evidence of health effects associated with exposures to ambient air pollutants is a difficult task. Although the effects of unmeasured atmospheric pollutants cannot be controlled for, in this study we observed a progressive and persistent pattern of nasal epithelial damage, which was likely further enhanced by the fact that the subjects exercised during the exposure (*29*). The nasal epithelial damage alters normal physiological nasal functions, which in turn may increase toxic insults to lower airways. Additional research is needed to answer three relevant questions: How long does it take the nasal epithelium to recover? What is the nature and magnitude of the lower respiratory tract pathology? and What are the long-term effects, if any, upon the respiratory system of this brief exposure?

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